THE INFLUENCE OF SALINITY ON THE MAGNESIUM AND WATER FLUXES OF A CRAB

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The shore crab *Pachygrapsus crassipes* is known to be an osmotic regulator in both dilute and concentrated sea water, but its antennary glands are ineffective as organs of osmo-regulation inasmuch as the urine remains essentially isotonic to the blood, regardless of the salinity of the external medium (Jones, 1941; Prosser *et al.*, 1955; Gross, 1957a). On the other hand, Prosser *et al.* (1955) demonstrated Mg concentrations in the urine of this species when it is exposed to osmotic stresses, which suggests that the antennary glands are effective regulators of Mg. Gross (1959) confirmed these observations, but pointed out that the volume of urine produced, as well as the urine concentration of Mg, must be known before the antennary glands could be considered certain regulators of this cation. Prosser *et al.* (1955) also observed that while the urine concentration of Mg increased tremendously when the crab was immersed in increasingly saline media, the urine Na, contrary to expectation, decreased. They suggested that Na and Mg compete for transport across the membranes of the antennary gland with Mg predominating. When the crab was immersed in artificial Mg-free sea water equivalent to 170% of normal salinities, the observed concentration of Na in the urine was much higher than it was when the crab was immersed in 170% natural sea water, thus supporting the suggestion. However, the effects of such a treatment on the urine Mg concentration were not reported.

Even though *Pachygrapsus* is a strong regulator in large osmotic stresses, its blood tends toward concentrations which are intermediate between those it has in normal sea water and the concentration of the external medium (Jones, 1941; Prosser *et al.*, 1955; Gross, 1957a). Gross (1957a) demonstrated that such changes in the concentration of the blood are brought about by salts and not water; that is, the volume changes of the animal were insignificant. This must mean that either the formed tissues when bathed by such altered blood concentrations also remain unchanged in volume or that their volumes change at the expense of the blood space. Shaw (1955) demonstrated that the volume of muscle tissue in the hyper-regulating crab *Carcinus* increased when the animal was immersed in dilute sea water.

The present investigation will show that the efflux of Mg from *Pachygrapsus* is principally a function of water turnover and not due immediately to the Mg gradient between blood and external medium. It also will be shown that muscle tissue increases in volume when the crab is immersed in dilute sea water, and that it decreases in volume when the crab is immersed in concentrated sea water. This results in volume alterations in the blood space.

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Material and Methods

The lined shore crab, *Pachygrapsus crassipes* Randall, was collected at Dana Point, Laguna, and Ballona Creek, California. Only mature crabs of more than 15 grams were used. Care was taken that none was undergoing moult.

Blood and urine were sampled as previously described; analyses of Na and K were made by flame photometry and Ca and Mg by titration with ethylene diamine tetra acetic acid (EDTA) (Gross, 1959).

Artificial sea water was prepared according to the tables of Barnes (1954), normal sea water being considered to contain the following concentrations (meq./l.) of the major ions: Na, 460; K, 10; Ca, 20; Mg, 104; Cl, 538; SO_4, 56. The pH was adjusted to 8.0. In the Mg-free media Na was substituted for the deleted Mg and where Mg was increased above normal, relative to the other ions, Na was deleted accordingly to attain the desired osmotic pressure.

The effect of abnormal concentrations of medium Mg on blood and urine ionic concentrations was studied in two ways: (1) Groups of crabs from 100% natural sea water were immersed into small volumes of Mg-free artificial sea water of salinities equivalent to 50%, 100% and 150% of natural sea water, and into media equivalent to 50% in salinity but containing Mg equal to that of 100% natural sea water (104 meq./l.). Also, one group was immersed in a medium equivalent to 100% sea water in total salinity, but which contained half again as much Mg (156 meq./l.). All animals first were rinsed in the respective test medium before immersion. After 24 hours, the blood and urine of the experimental animals were analyzed for Na, K, Ca and Mg; the media which were originally Mg-free were analyzed for Mg. In the small volumes of media used for this group, the crabs could rise partially out of the water. This kept the mortality rate low over the 24-hour period of immersion, thus permitting a study of ionic alterations in the blood and urine in the artificial media.

(2) The second group of experiments was conducted primarily to measure rates of Mg excretion. Here animals were placed in large volumes of medium to assure complete and uniform immersion throughout the test period. Crabs previously acclimatized for 24 hours in natural sea water of salinities respective to their subsequent test media were immersed in large volumes of Mg-free 50%, 100% and 150% artificial sea water, these, again, having been rinsed first in the test media. During acclimatization in natural sea water, the crabs could rise out of the water. Again after a period of immersion ranging from one to six hours the blood and urine of the animals were analyzed for Na, K, Ca and Mg and the medium was analyzed for Mg. All experiments were conducted in a temperature-controlled room at 15° C.

In order to determine the volume changes in the muscle tissue of *Pachygrapsus* under different osmotic stresses, leg muscle from animals which had been immersed for three days in 50%, 100% and 150% natural sea water was rinsed in isotonic glucose and blotted uniformly. (Glucose concentrations were calculated from the tables in Gross, 1959.) These were then weighed and dried to constant weight in a drying oven at 95° C. The difference between dry weight and wet weight was considered to be the water content of the tissue.

Changes in the blood space volume were shown by calculating the volume of distribution of C^{14}-tagged sucrose in the blood space one minute after injection. Crabs removed from immersion for three days in 50%, 100% and 150% natural sea

water were injected at the base of the fourth walking leg with 0.3 to 0.5 ml. (depending on the size of the crab) of 1 *M* sucrose which had been tagged with C^{14}.

About one minute after the injection, a sample of blood was taken from the opposite side of the animal and diluted with 5 ml. of 1 *M* untagged sucrose. The quantity of blood was determined by weight and averaged about 0.3 gram. Then a 0.10-ml. aliquot of the diluted blood was absorbed onto a filter paper disc, allowed to dry and counted on a Nuclear-Chicago scaling unit. Mean counts thus obtained for three discs made from each diluted blood sample were compared with the mean counts of three 0.10-ml. aliquots of the dose which were plated in the same manner. Since the blood was diluted in 1 *M* sucrose and the dose was 1 *M* sucrose, the error

TABLE I

Ion concentration in the urine of Pachygrapsus *immersed in small volumes of artificial sea water*

		50%			100%			150%		
		Mean	S.D.	No.	Mean	S.D.	No.	Mean	S.D.	No.
Na (meq./l.)	N	380	60	37	378	64	15	353	106	30
	0	400	28	12	332	96	12	430	81	15
	+	401	33	25	319	80	8			
K (meq./l.)	N	10.0	3.5	37	7.8	1.4	15	9.6	1.1	30
	0	14.3	8.7	12	15.9	8.0	12	16.0	4.2	15
	+	11.0	3.8	25	7.0	2.0	8			
Ca (meq./l.)	N	32.7	7.1	31	36.0	6.3	15	47.9	5.2	20
	0	34.5	4.5	13	34.5	4.5	13	47.6	18.6	15
	+	31.1	8.7	19	37.0	7.8	14			
Mg (meq./l.)	N	70.5	41	31	305	130	15	408	122	29
	0	85.0	42	16	298	104	13	281	85	15
	+	65.5	23	18	368	101	14			

N = crabs immersed in concentrations of natural sea water.
0 = crabs immersed in Mg-free sea water.
+ = crabs immersed in artificial sea water containing abnormally high Mg: in 50% sea water = 104 meq./l.; in 100% sea water = 156 meq./l.

due to self-absorption should be essentially the same in radio-assays of both blood and dose. Care was taken to assure uniform geometry. At least 1000 counts were observed for each sample, and maximum rates did not allow significant coincidence.

The volume of distribution for sucrose in one minute was, therefore, calculated from the equation: $V = \frac{d/b}{w} \times 100$

where V = volume of distribution of sucrose in one minute (% body weight);
d = observed total activity (counts/min.) injected into the crab;
b = observed activity (counts/min.) per gram of blood;
w = weight of crab (g.).

Also, a correction was applied for the volume of the dose.

After the blood samples were taken, the crabs were placed in a closed chamber

containing a $Ba(OH)_2$ trap designed to absorb CO_2. Radio-assay of the total precipitate thus collected in 24 hours for ten crabs demonstrated no significant activity, suggesting that the sucrose was not metabolized. It is possible that some was fixed in the body, but it seems more likely that it remained in solution in an unchanged state at least for the brief period (one minute) during which the dilution was being observed.

Results

Table I compares the urine Mg of crabs immersed in small volumes of artificial sea water with the urine Mg of crabs immersed for 24 hours in small volumes of natural sea water. The value for crabs in 100% natural sea water is higher than previously reported, but is believed to be more reliable because of improved technique in sampling the urine; the values for crabs in 50% and 150% natural sea water have been reported previously (Gross, 1959). It is clear from these data that when *Pachygrapsus* is immersed in 50% and 100% Mg-free artificial sea water, the concentration of Mg in its urine is close to the concentration of Mg in the urine of crabs immersed in the same salinities of natural sea water. When a crab is immersed in 50% sea water for 24 hours, the Mg concentration of its urine is about the same whether the medium Mg is 0, 52 or 104 meq./l. In salinities of 100% normal, the observed mean concentration for urine Mg was about the same for animals immersed in Mg-free water as for those from natural sea water; for animals immersed in 100% artificial sea water containing abnormally high Mg, the urine Mg was slightly higher than for crabs from 100% natural sea water (368 meq./l. to 314 meq./l., respectively), but these means are not significantly different (Table I). The urine Mg of crabs immersed in small volumes of Mg-free 150% sea water was less than that for crabs from 100% or 150% natural sea water. This is difficult to interpret, but possibly could be explained as a reflection of the crab's greater tendency to remain out of the artificial medium. Such an argument is supported by the fact that the urine Mg for crabs immersed in large volumes of Mg-free 150% sea water averaged about the same (470 meq./l.) as did crabs from small volumes of 150% natural sea water (408 meq./l.). Also, when the crabs were immersed in large volumes of 100% Mg-free sea water, the urine Mg was about the same as for crabs immersed

Table II

Excretion of Mg by Pachygrapsus *in stress media*

Test medium	Medium volume*	Mean urine Mg meq./l.	S.D.	No.	Mean Mg excreted meq./day/g.	S.D.	No.
150% sea water without Mg	small (24 hrs.)	281	85	15	0.0021	0.001	14
	large (6 hrs.)	471	152	17	0.0073	0.0054	18
100% sea water without Mg	small (24 hrs.)	298	104	13	0.0070	.0033	14
	large (6 hrs.)	243	103	15	0.0095	0.0084	15
50% sea water without Mg	small (24 hrs.)	85.0	42	16	0.0066	0.0030	20
	large (1 hr.)	72.0	44	12	0.0433	0.022	12

* Time in parentheses = period of immersion.

in 100% natural sea water (Tables I and II). Table III presents probability values for analyses of Mg excretion.

It might be argued that the animals immersed in the larger volumes of artificial sea water were previously acclimatized to the respective salinities, and that sufficient time had not been given to permit alteration of the urine concentrations. However, when 13 crabs were transferred directly from 100% natural sea water and immersed for six hours in a large volume of 150% Mg-free sea water, the mean urine Mg was 713 meq./l., indicating not only that considerable changes can occur in the urine Mg concentration in six hours, but also that the greater hyperosmotic stress due to transferring the animals directly from 100% natural sea water to 150% artificial sea water probably made the urine even more concentrated with respect to Mg, again independently of the influx of this ion from the medium. Thus, for the periods

TABLE III

Probability values for analyses of Mg excretion

A. Comparison of urine Mg concentrations: crabs immersed in artificial sea water vs. crabs immersed in natural sea water

	50% Sea water		100% Sea water		150% Sea water	
	Large volume	Small volume	Large volume	Small volume	Large volume	Small volume
Mg-free	>0.50	>0.20	=0.20	>0.50	>0.10	<0.001
Excess Mg		>0.50		>0.20		

B. Comparison of rates of Mg loss by crabs completely immersed in Mg-free artificial sea water

100% S.W. vs. 150% S.W.	>0.30
100% S.W. vs. 50% S.W.	<0.001
150% S.W. vs. 50% S.W.	<0.001

indicated the concentration of Mg in the urine is not determined by the influx of this ion from medium, but rather, at least indirectly, by the osmotic pressure of the external medium.

Table I also compares the urine concentrations of the other three major cations of the crabs immersed in small volumes of artificial sea water with those immersed in the respective salinities of natural sea water. Contrary to the findings of Prosser *et al.* (1955), there is no dramatic increase in urine Na when Mg is deleted from the medium. Although in 100% artificial sea water with high Mg the urine Na of *Pachygrapsus* was somewhat low, this could be accounted for by the low Na in the medium rather than the high Mg. Then in 150% Mg-free sea water the urine Na was high, but again this was likely due to the high Na in the medium, substituting for the deleted Mg. The concentration of Ca in the urine also seems unaffected by the absence or relative increase of Mg. With regard to K, the urine concentrations of this ion are significantly higher when the animal is immersed in 100% and 150% Mg-free sea water, than when immersed in the same concentrations of natural sea water ($P < 0.01$). The mean urine K for animals immersed in 50% Mg-free sea water also was higher than for crabs immersed in 50% natural sea water, but the difference cannot be shown to be significant. Neither is the urine K of animals

immersed in 50% sea water with normal Mg concentrations (104 meq./l.) significantly different from that of crabs from 50% natural sea water.

Table II reveals the rate of Mg excretion in the different Mg-free salinities. Thus, considering only those crabs immersed in the large volume where they could not rise out of the water, it can be seen that the mean Mg excreted in 100% sea water is greater than the mean Mg excretion in 150% sea water, but the difference between these means is not significant (Table III). On the other hand the rate of Mg loss is four times as great in 50% sea water as it is in normal sea water; this difference is highly significant, $P < 0.001$ (Table III). Table II also shows that less Mg is lost to a small medium than to a large one. This, of course, would be expected because the animals could rise out of the small volume. Among all test media the difference in rates of Mg excretion between large and small volume treatments was smallest for 100% sea water, suggesting that in this salinity there is a minimum attempt to rise out of the water. The tendency for this crab to avoid an osmotic stress has been noted previously (Gross, 1957b).

The concentration of Mg in the urine of crabs immersed in large volumes of artificial sea water also is given in Table II. It will be recalled that these crabs first were acclimatized to the respective salinities of natural sea water before treatment in artificial sea water. Thus, there was no large change in the osmotic gradient to which the animal was subjected following acclimatization. The indicated periods of immersion were chosen because after such time in the test media, the urine Mg did not differ significantly from that of crabs removed from the respective acclimatizing salinities of natural sea water. Immersion periods of more than one hour in large volumes of Mg-free 50% sea water apparently deplete the Mg supply of the crab and the urine Mg becomes greatly reduced in concentration. Mg excretion of *Pachygrapsus* immersed for one hour in all three salinities of Mg-free sea water could not be compared because in this brief time insufficient amounts of the ion were released in 100% or 150% sea water to be detected with precision by the methods available.

If, then, the consistent concentration of Mg in the urine were known throughout a period of immersion and the amount of Mg lost to the medium in that period also were known, then assuming that the antennary glands are the sole pathways of Mg efflux, the volume of urine necessary to excrete the observed loss of Mg can be calculated. Estimations of urine flow from mean rates of Mg loss and mean Mg concentrations in the urine follow: in 150% sea water the volume of urine production was calculated to be 1.5% body weight/day; in 100% sea water, 3.9% body weight/day and in 50% sea water, 58% body weight/day.

The value for *Pachygrapsus* immersed in 100% sea water compares favorably with values reported by Webb (1940) on *Carcinus* and by Robertson (1939) for *Cancer* when the crabs were immersed in normal sea water, but only half the value reported by Nagel (1934) for *Carcinus* immersed in normal sea water. These workers, however, plugged the nephropores and assumed the gain in weight was due to urine which could not escape. Values obtained by three different methods on the prawn, *Palaemonetes,* in 100% sea water were more than twice as large as the above rate for *Pachygrapsus* (Parry, 1955).

As might be expected, the calculated rate of urine flow for *Pachygrapsus* was less in 150% sea water than in 100% sea water and the rate in 50% sea water

greater than in normal sea water, but of such magnitude (58% body weight/day) that it is subject to question. This rate was determined on the basis of Mg excretion after one hour total immersion, but since the crabs had been acclimatized to 50% natural sea water before treatment in the Mg-free medium, there was no large increase in the osmotic gradient between external medium and the blood of the animal. However, forced total immersion, which did not take place in the acclimatizing procedure, caused more surface of the crab to be exposed to stress and this probably caused an increase in the water influx and the consequent increase in urine flow. It should be emphasized again that the concentration of Mg in the urine following immersion in large volumes of 50% Mg-free sea water averaged about the same as for crabs immersed for 24 hours in 50% natural sea water. It is thus likely that the urine Mg concentration for this group of crabs did not change during the one-hour immersion period. Either, then, the rate of urine flow for crabs thus treated is as calculated (58% body weight/day) during that period of immersion, or in such a hypotonic medium, mechanisms of Mg loss are different from those in crabs immersed in 100% and 150% Mg-free sea water. The Mg gradient between blood and external medium (Mg-free) would be about the same for all three conditions; yet Table II demonstrates the greatest mean loss to 50% and the smallest mean loss to 150% sea water. It is our opinion that if the principal pathway for Mg loss in all the above conditions were the antennary glands, then the above value for the rate of urine flow is a fair approximation for the conditions described. It would follow that such a rate could not be sustained, and it is interesting that the number of fatalities for crabs immersed for one hour in the large volume of 50% sea water was twice as large as the combined number of fatalities for crabs totally immersed for six hours in 100% and 150% sea water. Also, urine Mg in a few crabs which survived for six hours in 50% Mg-free sea water was essentially nil, and the actual amount of Mg lost to the medium was about twice that lost by crabs immersed in large volumes of Mg-free 100% sea water for the same period. This indicates exhaustion of the Mg supply in the crabs. When nine crabs which had been acclimated for 24 hours in small volumes of 50% natural sea water were transferred to large volumes of the same medium, for six hours, the mean urine Mg dropped to 47.2 meq./l., S.D. = 13.4. This is significantly less than the urine Mg of crabs from small volumes of 50% natural sea water (Table I): $P = 0.01$. It is suggested that sudden total immersion in natural 50% sea water causes a depletion of Mg reserves more rapidly than they can be replenished from a medium of this salinity. The low mortality rate in this group of crabs also suggests that Mg depletion is a cause of death in the crabs immersed in Mg-free 50% sea water, but also raises the question as to how long *Pachygrapsus* can survive totally immersed in 50% sea water in nature.

In order to estimate the urine flow when *Pachygrapsus* was removed from water, ten crabs were taken from normal sea water, rinsed in distilled water to wash away residual salts, then blotted dry. The crabs then were placed in dry containers and kept in a relatively humid temperature-controlled room at 15° C. for 72 hours. Then the crabs and their containers were rinsed with distilled water and the washings saved for Mg analysis. Also, the urine from these animals was sampled and analyzed for Mg. The urine concentration for Mg and the total excretion of Mg into the container then should yield the volume of urine flow

during the 72-hour period. The average calculated rate of urine produced, thus determined, was 0.02% body weight/day, which is hardly significant.

Table IV demonstrates the effects of altered Mg in the medium on the blood concentration of the four major cations in *Pachygrapsus*. As would be expected, the blood Mg concentrations of crabs immersed in 100% and 150% Mg-free sea water are less than those of crabs from the same salinities of natural sea water. However, the blood Mg of crabs immersed in Mg-free 50% sea water was not

TABLE IV

Ion concentrations in the blood of Pachygrapsus *immersed in small volumes of artificial sea water*

		50%			100%			150%		
		Mean	S.D.	No.	Mean	S.D.	No.	Mean	S.D.	No.
Na (meq./l.)	N	397	24	37	483	17.3	36	582	34	30
	0	410	30	15	493	9.6	14	562	40	15
	+	410	30	21	478	15.1	8			
	N/0	0.97			0.98			1.04		
	N/+	0.97			1.01					
K (meq./l.)	N	7.36	1.4	37	7.43	0.72	36	10.2	1.5	30
	0	5.78	1.0	15	9.19	0.82	14	8.62	1.4	15
	+	6.53	1.0	21	7.88	1.4	8			
	N/0	1.27*			0.81*			1.19*		
	N/+	1.13			0.94					
Ca (meq./l.)	N	34.8	7.9	24	29.6	5.9	44	36.4	4.8	30
	0	33.1	9.6	14	32.0	8.1	14	40.5	6.3	15
	+	29.7	6.4	16	38.1	8.4	17			
	N/0	1.05			0.93			0.90		
	N/+	1.17			0.78*					
Mg (meq./l.)	N	13.6	5.4	24	20.0	6.1	44	27.1	4.2	30
	0	13.2	5.1	14	11.7	5.7	14	16.7	7.5	15
	+	19.7	7.4	16	33.2	6.1	15			
	N/0	1.03			1.71*			1.62*		
	N/+	0.69*			0.60*					

N = crabs immersed in natural sea water.
0 = crabs immersed in Mg-free artificial sea water.
+ = crabs immersed in artificial sea water containing abnormally high Mg: in 50% sea water = 104 meq./l.; in 100% sea water = 156 meq./l.
* = significantly different from unity: $P < 0.01$.

significantly less than the blood Mg for crabs from 50% natural sea water. On the other hand, blood Mg for crabs immersed in 50% artificial sea water which contained normal Mg (104 meq./l.) was about equal to the blood Mg of crabs from 100% natural sea water (20 meq./l.). Also, the mean blood Mg of crabs immersed in 100% artificial sea water with high Mg (156 meq./l.) was 33.2 meq./l., which is significantly higher ($P < 0.01$) than the concentration of this ion for crabs which had been immersed in either 100% or 150% of natural sea water (20.0 and 27.1 meq./l., respectively). This is particularly interesting because as

indicated above, the urine Mg of crabs from this artificial medium is about equal in concentration to that of crabs from 100% natural sea water. It is apparent that the blood concentration of Mg is influenced by the influx of Mg from the medium, even though the Mg concentration in the urine is not directly affected.

Table IV also shows that blood Na is essentially unaltered by abnormal concentrations of Mg in the medium. It is interesting, however, that when immersed in 100% artificial sea water containing high Mg, the blood Na of *Pachygrapsus* remains normal, even though the concentration of this ion in the medium was reduced because of the high Mg. Blood Ca is unaltered in all conditions except when the crab is immersed in 100% sea water with high Mg. Here the blood Ca is significantly higher ($P < 0.01$) than for crabs from 100% natural sea water. There seems to be an interdependence between the regulation of Ca and Mg under these conditions. In Mg-free 50%, 100% and 150% sea water the blood K differs from the blood K of crabs from natural sea water. There is no consistent trend,

TABLE V

Apparent volume of distribution of sucrose in the blood space and water content for muscle in* Pachygrapsus *following immersion in different salinities*

Test medium (% sea water)	Mean volume of distribution (% body wt.)	S.D.	No.	Mean water content of muscle (% wet wt.)	S.D.	No.
50	15.4	1.39	10	76.58	1.77	18
100	18.7	3.45	11	75.00	1.40	25
150	26.7	5.34	14	71.70	1.71	34

* One minute after injection.

but it can be suggested that there also is an interdependence between the regulation of Mg and K. Such a suggestion is supported by the above mentioned differences in urine K between animals from Mg-free sea water and those from natural sea water.

Table V demonstrates that the muscle tissue of *Pachygrapsus* gains water when the animal is immersed in 50% sea water and loses water when it is immersed in 150% sea water. That is, the muscle is permeable to water in both directions. This is particularly interesting inasmuch as the animal itself shows no significant weight changes during such treatments (Gross, 1957a). Table V also shows that the calculated apparent volume of distribution for sucrose one minute after injection into the blood space is smallest when the crab is in 50% sea water and largest when the crab is immersed in 150% sea water. This is interpreted to mean that the blood space volume of *Pachygrapsus* is altered when the animal is transferred from one salinity to another by the changing volume of the formed tissues. There may be objections to the use of only one concentration of sucrose for the injected dose (1 *M*), but the volume of the dose was no greater than 0.5 ml. and, if anything, would be expected to cause an increase in blood volume for crabs from dilute sea water and a reduced blood volume for crabs from concentrated sea water.

Discussion

The diagram presented in Figure 1 suggests the course of events with respect to Mg and water fluxes when *Pachygrapsus* is immersed in different concentrations of sea water. Since the indicated Mg values are based on the crab's response to Mg-free media, it can be seen that the concentration of Mg in the urine of

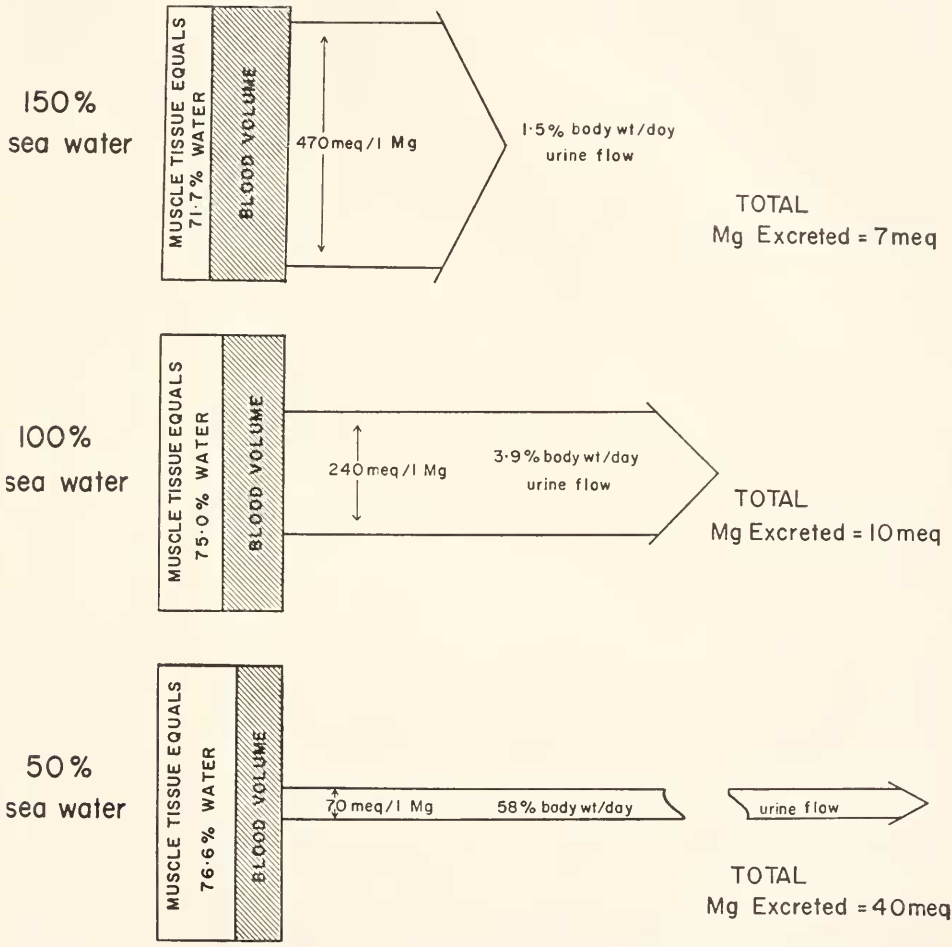

Figure 1. Scheme of urine flow, water shifts and Mg effluxes in *Pachygrapsus* when exposed to different osmotic situations (indicated by the concentration of the external medium in % sea water on left side of diagram). Arrows which represent urine flow and Mg efflux are based on mean values for Mg concentrations in the urine and Mg losses to the medium when the crab is completely immersed in 50%, 100% and 150% Mg-free artificial sea water (see Table II). Length of arrow = volume of urine flow (% body weight/day); width = urine concentration of Mg (meq./l.); arrow area = relative amount of Mg excreted (meq.). Rectangles which are not drawn to scale represent crabs and illustrate the differences in blood volume (hatched area) in different osmotic situations, as suggested by the calculated volume of distribution for sucrose (Table V), and the volume of formed tissue (blank area), as suggested by the water content of muscle for crabs from different osmotic situations (Table V).

Pachygrapsus is directly related to the salinity of the external medium and not to the concentration of Mg in the medium or its influx into the animal. Likewise, it is shown that the efflux of Mg is inversely related to the concentration of this ion in the urine. (While the mean Mg loss in 150% was less than the mean Mg loss in 100% sea water, these values are not significantly different. However, the mean Mg loss in 50% sea water was more than four times the loss in the other two media.) Assuming that the antennary glands are the principal pathways for Mg efflux for all conditions, then small volumes of urine are produced in concentrated sea water and large volumes of urine are produced in dilute sea water. Figure 1 likewise shows that when *Pachygrapsus* is immersed in dilute sea water the water content of formed tissues is higher than when it is immersed in normal sea water, and conversely when the crab is immersed in concentrated sea water the water content of its formed tissues is less than when it is immersed in normal sea water. Such changes in water content effect volume alterations in the tissues at the expense of the blood space. As shown in Figure 1, then, the concentration of urine Mg is apparently determined by the water flux and is immediately independent of the concentration of this ion in the medium. Also, it is relatively independent of the Mg concentration in the blood. It will be observed (Table IV) that the blood Mg of crabs immersed in Mg-free sea water can be reduced below normal, yet the urine Mg (Table I) remains high (*e.g.* in 100% sea water). Again, in crabs immersed in 100% artificial sea water with high Mg the blood Mg becomes elevated above normal, but the urine Mg remains essentially the same as it is in crabs from 100% natural sea water. This response is contrary to the findings of Webb (1940), who demonstrated in *Carcinus* in a similar experiment that increases in the concentration of Mg in the external medium were reflected in the urine, but very little in the blood. Thus the mechanism of Mg regulation in *Pachygrapsus* may be fundamentally different from that of *Carcinus*. In the latter case the concentration of Mg in the urine increased even though the total salinity of the medium was normal; yet the blood Mg remained close to the concentration it attains in crabs from 100% natural sea water. As proposed by Webb (1940), excretion of Mg in *Carcinus* by way of the antennary glands does depend on the influx of this ion from the medium and consequently on the concentration of Mg in the blood. On the other hand, in *Pachygrapsus,* as shown above, the concentration of Mg in the urine is relatively independent of both the influx of this ion from the external medium and the concentration of Mg in the blood *per se*.

It seems, then, that in *Pachygrapsus* the concentration of urine Mg depends inversely upon the rate at which the antennary glands form urine or the rate at which the Mg-containing fluid is transported across the membranes of the antennary glands. This, in turn, depends on the magnitude and direction of water flux between the animal and its external medium.

The volume of urine flow calculated from the rates of Mg excretion seems reasonable for crabs immersed in 100% sea water. At least this value (3.9% body weight/day) agrees favorably with other values in the literature on other species, suggesting that this may be a valid method for estimating urine production. It is interesting that in a crab completely immersed in 150% sea water the calculated urine flow is 1.5% body weight/day, because there is a tendency for the crab to lose water to the hypertonic medium, yet there is no significant weight change

even though fluid is being lost by way of the urine and probably by diffusion. The animal, therefore, must possess a mechanism for actively taking up water. Drinking is suggested as the principal method for replacing lost fluid. Burger (1957) has demonstrated drinking in *Homarus;* Green *et al.* (1959) have produced evidence that the gut takes part in the hypo-osmotic regulatory mechanism of *Uca.*

The excessive calculated urine flow for crabs completely immersed in 50% sea water (58% body weight/day) is difficult to interpret. It does not seem that such a rate could be sustained for long. This must mean that should this species inhabit water of such low salinities for prolonged periods, it must either alter its permeability or perhaps, being free to come out of the water, limit its period of immersion. The calculated rate of urine flow in the small volume of 50% sea water where the crab could rise out of the water was less than 10% body weight/day.

Again it becomes clear that the concentration alone of a given ion in the urine does not reveal the relative rate of excretion of that ion or, in the case of crabs, the relative quantitative role of the antennary glands in regulation of a particular substance. Table II reveals that the greatest mean loss of Mg was by crabs immersed in 50% sea water; yet these same crabs possessed the lowest concentrations of Mg in the urine. Conversely, the smallest mean loss of Mg was by the crabs immersed in 150% sea water which in turn had the highest Mg concentrations in the urine (Table II, Figure 1). In the case of crabs kept out of the water, the concentration of urine Mg was high (300 meq./l.), yet the amount excreted in three days could hardly be measured.

It is suggested from the lack of Mg loss when the crab is out of the water that *Pachygrapsus* must depend upon an uptake of water in order to excrete urine. This, then, is another physiological limitation binding this semi-terrestrial species to the sea. Gross (1955) discusses other characters which limit the terrestrial life of this crab.

It has long been recognized that when an aquatic animal enters a medium of a different salinity, it must undergo certain physiological adjustments which include the mechanisms of tolerance permitting adequate functioning of the cells and tissues despite changes in the salt concentration of the surrounding body fluids, or regulation which keeps those changes in blood concentrations at a minimum. Evidence has been produced by the present investigation that *Pachygrapsus* is capable of regulating the total water content of its body by expelling the excessive influx when in a hypotonic medium by a rapid flow of urine and, conversely, compensating in some way for the physical efflux of water when immersed in a hypertonic medium (perhaps by drinking). Table V, however, shows that the muscle tissue cannot regulate its volume, at least not during a three-day exposure to osmotic stress, the result being that the anatomy of the vascular system also becomes altered. It does not seem that the changes in the volume of the blood space suggested by data in Table V would not affect the efficiency of the animal.

It would seem, therefore, that should *Pachygrapsus* inhabit salinities which vary much from those of normal sea water for prolonged periods, it would be obliged to control the volume of its formed tissues so that a normal and efficient anatomy could be assured for the vascular system.

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Summary

1. The concentration of Mg in the urine of *Pachygrapsus* is dictated by the salinity of the external medium and not by the Mg concentration in that medium or by the rate of Mg influx from the medium into the animal. Thus, during brief periods of immersion in 50%, 100% or 150% sea water the urine Mg concentration will reflect the salinity of the medium, irrespective of whether Mg is absent or in abnormally high concentrations.

2. The Na concentration in both blood and urine is not drastically altered by abnormal Mg levels in the external medium of any salinity tested.

3. After immersion in Mg-free 100% and 150% sea water the urine K of *Pachygrapsus* is higher than it is after immersion in the respective concentrations of natural sea water. Urine K is not influenced by the Mg concentration of 50% artificial sea water or by abnormally high Mg in 100% sea water. Blood K concentrations are affected by varying concentrations of Mg in the external medium of both dilute and concentrated salinities, but there is no definite trend.

4. The concentration of Ca in the urine of *Pachygrapsus* is unaffected by the Mg levels of all salinities tested. Blood Ca was not observed to be altered by abnormally high or low Mg levels in all media tested except in 100% artificial sea water with high Mg (156 meq./l.), where the blood Ca was significantly higher than for animals from 100% natural sea water.

5. While the concentration of urine Mg is not determined immediately by the influx of this ion into the animal, the blood Mg concentration is lowered when the crab is immersed in a Mg-free medium and raised when the medium Mg is abnormally high. The concentration of urine Mg is relatively independent of the levels of Mg in the blood.

6. *Pachygrapsus* excretes more Mg in 50% sea water than in 100% sea water and perhaps less in 150% sea water than in 100% sea water, even though the concentration of Mg in the urine is in the reverse order (*i.e.*, 150% > 100% > 50%).

7. Calculated rates of urine production for *Pachygrapsus* completely immersed in different salinities follow: in 150% sea water, 1.5% body weight/day; in 100% sea water, 3.9% body weight/day; in 50% sea water, 58% body weight/day. The observed rate for crabs immersed in 50% sea water is not believed to be sustained for long, as suggested by the high mortality rate. When removed from water, the volume of urine excreted by *Pachygrapsus* is insignificant.

8. The concentration of urine Mg in *Pachygrapsus* thus is inversely related to the rate at which urine is produced by the antennary glands and this is dependent on the magnitude and direction of the water flux, imposed by the physical gradient between the crab and its external medium.

9. The volume for muscle tissue in *Pachygrapsus* increases when the crab is transferred from normal sea water to dilute sea water and decreases when it is transferred to concentrated sea water. Such volume changes take place at the expense of the blood space.

10. It is suggested that alterations in the volume of the blood space caused by osmotic stress likely would reduce the efficiency of the vascular system which in turn would impose further ecological limitations on this species.

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